



Isolation and study of the hormone Phoenixin-14 from the serum of obese women with polycystic ovary syndrome

Amaal M. Ali, Layla A. Mustafa

Chemistry department, Mosul university, Mosul, Iraq

amaalmohammed15@gmail.com

layal.abdulla@uomosul.edu.iq

Abstract:

Phoenixin (PNX) is a newly discovered neuropeptide that has been linked to reproductive function, It cleaved from the small integral membrane protein 20(SMIM20). The study aimed to isolation phoenixin-14 (PNX-14) from the blood serum of obese women with polycystic ovary syndrome (PCOS) and estimating its approximate molecular weight. Twenty-six milliliters of blood serum was collected infected with PCOS from Al-Salam Teaching Hospital in Mosul city, Iraq. For the period from the (first of July 2022 until the end of September 2022). The study included (50) samples of obese women with PCOS and (50) samples of women as healthy group, whose ages ranged between (16 years and above). Assessing the level of phoenixin-14 in the blood serum of women who did not suffer from polycystic ovary syndrome, as well as healthy and obese women with polycystic ovary syndrome, The results showed that the normal range of phoenixin-14 (99.08 ± 3.66 pg/mL) in the healthy group, the results showed a significant increase in the level of phoenixin-14 of obese women with polycystic ovary syndrome compared to healthy people and increase with increasing age and increase body mass index and also married compared to without married women. Results: the molecular weight of phoenixin-14 (1583.78 mol) and the molecular weight of phoenixin-14 obtained by gel filtration chromatography is (1697 mol), when using the (HPLC) technique, we found that the retention time of standard funkycin-14 is similar to the retention time of funkycin-14 (paek A) isolated and purified from the blood serum of obese women with PCOS.

KEYWORDS: Phoenixin-14, Isolation, PCOS, LH, GnRh.

عزل ودراسة هورمون الفونكسين -14 من مصل دم النساء البدينات المصابات بمتلازمة المبيض المتعدد الكيسات

آمال محمد علي، ليلي عبد الله مصطفى

قسم الكيمياء، جامعة الموصل، الموصل، العراق.

amaalmohammed15@gmail.com

layal.abdulla@uomosul.edu.iq

خلاصة:

الفونكسين (PNX) هو ببتيد عصبي مكتشف حديثاً تم ربطه بالوظيفة الإنجابية، وهو منفصل من بروتين الغشاء الصغير المتكامل 20 (SMIM20). هدفت الدراسة إلى عزل الفونكسين -14 (PNX-14) من مصل الدم للنساء البدينات المصابات بمتلازمة المبيض المتعدد الكيسات (PCOS) وتقدير وزنه الجزيئي التقريبي. تم جمع (26 مل) من مصل الدم المصابات بمتلازمة المبيض المتعدد الكيسات من مستشفى السلام التعليمي في مدينة الموصل، العراق، للفترة من (الأول من يوليو 2022 حتى نهاية سبتمبر 2022). شملت الدراسة (50) عينة من النساء البدينات المصابات بمتلازمة المبيض المتعدد الكيسات و (50) عينة من



النساء كمجموعة صحية، تراوحت أعمارهن بين (16 سنة فأكثر). تقييم مستوى الفونكسين-14 في مصل الدم لدى النساء اللواتي لم يعانين من متلازمة المبيض المتعدد الكيسات ، وكذلك النساء الأصحاء والبيديئات المصابات بمتلازمة المبيض المتعدد الكيسات ، أظهرت النتائج أن المعدل الطبيعي للفونكسين-14 (3.66 ± 99.08 بيكوغرام / مل) في المجموعة السليمة، أظهرت النتائج زيادة معنوية في مستوى الفونكسين-14 لدى النساء البيديئات المصابات بمتلازمة المبيض المتعدد الكيسات مقارنة بالأشخاص الأصحاء وزيادة مع تقدم العمر وزيادة مؤشر كتلة الجسم وأيضا في المتزوجات مقارنة بالنساء الغير متزوجات. النتائج: الوزن الجزيئي للفونكسين-14 (1583.78 مول) والوزن الجزيئي للفونكسين-14 الذي تم الحصول عليه بواسطة كروماتوغرافيا الترشيح الهلام هو (1697 مول)، عند استخدام تقنية (HPLC) ، وجدنا أن زمن الاحتجاز للفونكسين 14 القياسي مشابه لزمن الاحتجاز للفونكسين-14 (القمة A) المعزول والمنقى من مصل الدم لدى النساء البيديئات المصابات بمتلازمة المبيض المتعدد الكيسات.

KEYWORDS: Phoenixin-14, Isolation, PCOS, LH, GnRh .

Introduction

Phoenixin (PNX) is a newly discovered neuropeptide that has been linked to reproductive function, in both the hypothalamus and pituitary. PNX has two types or two more common forms, namely (PNX-14) and (PNX-20) [1]. It was discovered in the year 2013. It was discovered by a bioinformatics algorithm based on human genome report data used to predict nonspecific, highly conserved peptide sequences [2]. In the four years since then, phoenixin (PNX) has a clear role in reproduction and its receptor has been determined. In addition, there is some evidence that PNX may have some effects on the heart, nutrition, memory, and anxiety [3, 4]. (phoenixin-14) and 20 are two isoforms with similar mechanisms of action and biological receptors [5]. Phoenixin is cleaved from the C-terminal 20 (Smim 20) trans membrane integral small protein, also known as C4orf52 [6]. Smim20 is a component of the cytochrome c oxidase complex intermediate mitochondrial translation regulatory group , which is involved in (cytochrome c oxidase) biogenesis, and stabilizes the COX1 subunit [7]. PNX-14 is a residual 14 peptide found in multiple species including humans, rats, mice, pigs, and dogs [8,9]. As for (PNX-20), it is a peptide consisting of 20 residues, which is (PNX-14) extended from the N-terminus and differs in one amino acid between the coding region of the human, canine or swine sequence [8]. The hormone phoenixin (PNX) also affects reproduction, as in vitro experiments on cells of the anterior pituitary gland showed that PNX stimulates gonadotropin secretion by increasing the expression of GnRH receptors[10]. Phoenixin is also an intra-ovarian factor, that stimulating the growth of ovarian follicular cells via the GRP173 receptor and protein kinase A (PKA) by accelerating human granulosa cell (Gc) proliferation and stimulating the secretion of estradiol (E2)[11]. PCOS is an endocrine disease characterized by a defect of the pituitary-ovarian axis, for this reason the serum PNX-14 level is affected by the presence of this syndrome. The synthesis and secretion of peripheral and central peptides are truly impaired in women with polycystic ovary syndrome, and the synthesis and release of several central and peripheral adipokines and peptides is impaired in PCOS [12,13].



Materials and methods

Blood samples were collected and isolated from a group of obese women with polycystic ovary syndrome (PCOS) and from a women as (healthy subjects), Where their ages ranged between (16 years and above) from Al-Salam Teaching Hospital in Mosu city, Iraq. Isolate the serum by placing the blood tubes in a centrifuge at (3000 rpm) for 15 minutes . Then the serum was taken and kept in clean, covered tubes at a temperature(-20°C) [14]. To be compared with (50) blood samples of healthy women who do not have this syndrome, and the information related to the pathogens was written in a specialized questionnaire form for this purpose.

Phoenixin -14 measurement with ELISA

The concentrations of Phoenixin-14 in serum was measured by based on sandwich enzyme-linked immune-sorbent assay (ELISA) using Human PNX-14 (Phoenixin-14) (ELISA) Kit (Wuhan Fine Biotech Co., immediately. This kit is used to measure the Phoenixin-14 concentration in serum, plasma, tissue homogenates and other biological fluids. The primary objectives of our study can be listed as follows:

1.To compare serum Phoenixin-14 levels of obes women with PCOS and healthy women.

2.To reveal the possible correlation between serum Phoenixin-14 levels, demographic, and metabolic parameters.

3.To compare serum levels of Phoenixin-14 from Married and unmarried PCOS patients.

Isolation , partial purification and estimate the approximate molecular weight of phoenixin-14:

By gel filtration chromatography:. 1

This technique was used to isolate and estimate its approximate molecular weight [15]. The gel sephadex (G-25) was used to fill the separation column with dimensions (75 x 1.3 cm), then the gel was placed at a height of (69 cm).

Use distilled water as a washing solution. The protein content was monitored by reading the absorption intensity at (280 nm). The parts of the study that divided the study with the highest concentration of phoenixin were collected and dried by design to obtain a protein substance in good shape. The hormone was compared to standared phoenix-14 solution.

2.Quantitative of protein:

Estimation of protein level in serum was estimated using the Follin-Lowry method modified by researchers (Schacterle and Pollack, 1973) used to determine total protein concentrations [16-17].

.Partial purification of phoenixin-14: 3



Hormone deposition process: The hormone precipitated from the patient's blood serum by the gradual addition of acetone at a saturation of (40:60 v/v) and at a temperature (4°C) with continuous stirring , The mixture is left for a full night in the refrigerator, after which it is separated using a refrigerated centrifuge at a speed of (13000Xg) for a period of (20min) [14].

sedimentation process:

The protein precipitate resulting from the cooled centrifugation process for a period of approximately (24 hours) was precipitated by acetone at a ratio of (40:60 v/v) and the addition process took place for one hour at a temperature of (4°C) [18].

Reverse phase liquid chromatography technique:

According to the method used by the researcher [19], which is used to find out the approximate molecular weight of the compounds.

Statistical analysis:

Data analysis was performed using Origin Pro 2021 (64-bit) . All results were expressed as the mean $\pm$ standard error (SE). The results were analyzed statistically using the T-Test to find a significant difference between the study groups, and the probability level ($p \leq 0.05$) is a significant difference [20].

Results and discussion:

Phoenixin(PNX) is a recently discovered neuropeptide that regulates appetite, pain sensation, and nerve cells in the reproductive system, the central nervous system, the circulatory system, and surrounding tissues .A significant increase was observed at the probability level ($p \leq 0.05$) in the level of phoenixin-14 (PNX-14) in the serum of obese women with polycystic ovary syndrome (119.45 ± 5.30 pg /mL) compared to its level in the healthy women (3.66 ± 99.08 pg/mL) and this agrees With what the researchers mentioned [21]. Due to the lack of adequate clinical studies, no clear comment can be made on the relationship between BMI and PNX-14. In patients with obese polycystic ovary syndrome, the synthesis and secretion of many peripheral and central peptides

will be affected [13,22] . Adipokines are involved in lipogenesis and also in the regulation of communication between adipose tissue and endocrine and reproductive organs[23]. PNX is secreted into both central and adipose tissue, but in polycystic ovary syndrome the synthesis and release of (PNX-14) is altered. Ullah et al [21] showed that there is an association between BMI and Serum Phoenixin levels women Patients with PCOS. It has also been shown that both mature adipo cyts and preadipocytes contain a large amount of the Phoenixin receptor GPR1732 [24 ,25]. As a result of binding to these receptors, PNX-14 will



convert former adipose cells into mature adipocytes and increase the formation of white adipose tissue [24,25] .

Table(1) The serum concentration of phoenixin-14 in patients with PCOS compared to the control group.

phoenixin-14 concentration(pg/mL)	Mean ±Standard error	Increase %
patient group(obes women with pcos)	119.45±5.30	20.56%
Healthy women	99.08±3.66	

Table (2) shows the steps of purifying the PNX-14 hormone. The specific concentration of the hormone phoenixin-14 became in the obtained range (6.59pg/mL) in blood serum, and the recovery ratio was (2), The number of purification times was (17). Figure 1 shows the isolation profile of PNX-14 purified by gel filtration chromatography, As shown we obtained two peakes.

Table (2) Purification steps for PNX-14 from blood serum of PCOS women.

Purification steps	Total volume (mL)	Protein total (mg)	total concentration For the PNX-14 hormone ((pg/mL	Specific concentration for the PNX-14 hormone(pg/mL)	The number of purification Times	percentage to restore%
Serum from pcos women	26	73.23	132	1.80	1	100
sedimentation by acetone 40:60v/v	14	32.28	63.6	1.97	1	48.1
The resulting A peak On the eparating column the gel filtration of sephadex G-25	14	2.89	30.4	10.5	6	23.0

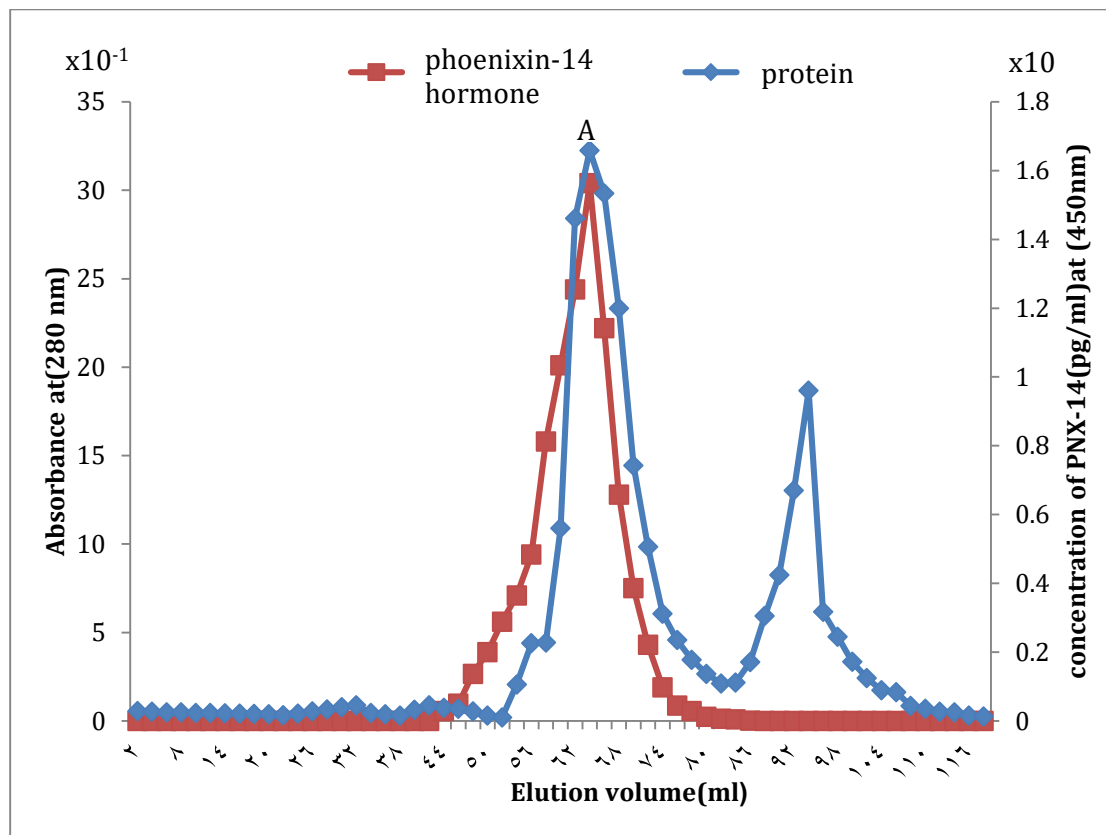


Figure 1: Purification of PNX-14 by gel filtration chromatography using sephadex G-25.

Estimation of approximate molecular weight .

Gel filtration chromatography is used to separate and estimate the approximate molecular weight. As shown in Figure 1, The elution volume of the PNX-14 solution collected by gel filtration column was (68mL), which corresponds to a molecular weight of (1629.29mol/L) when using the standard curve shown in Figure 2.

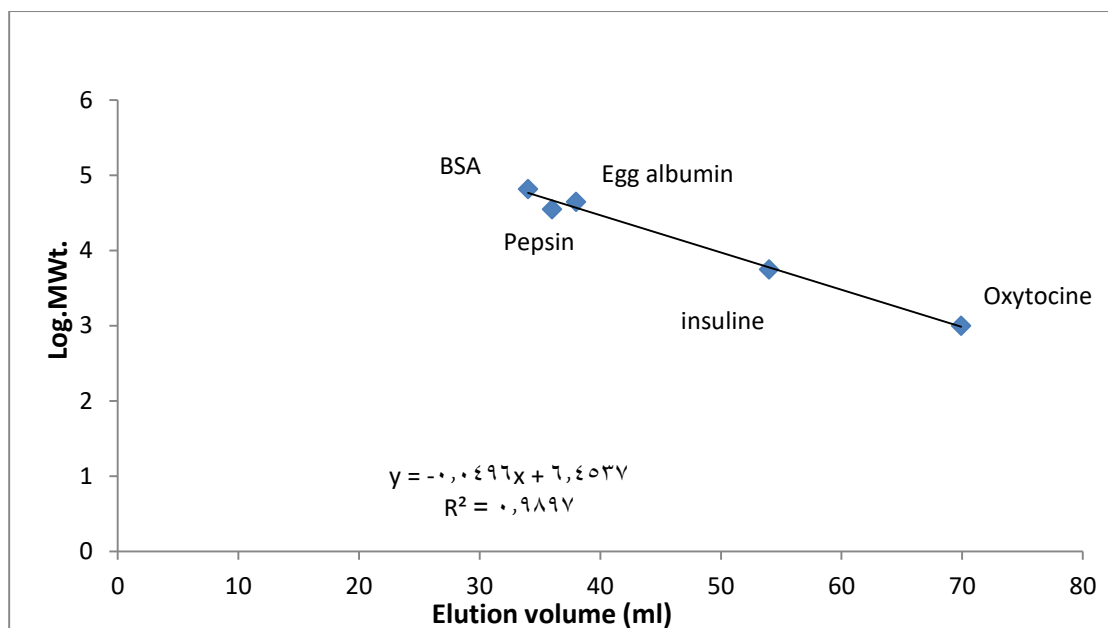
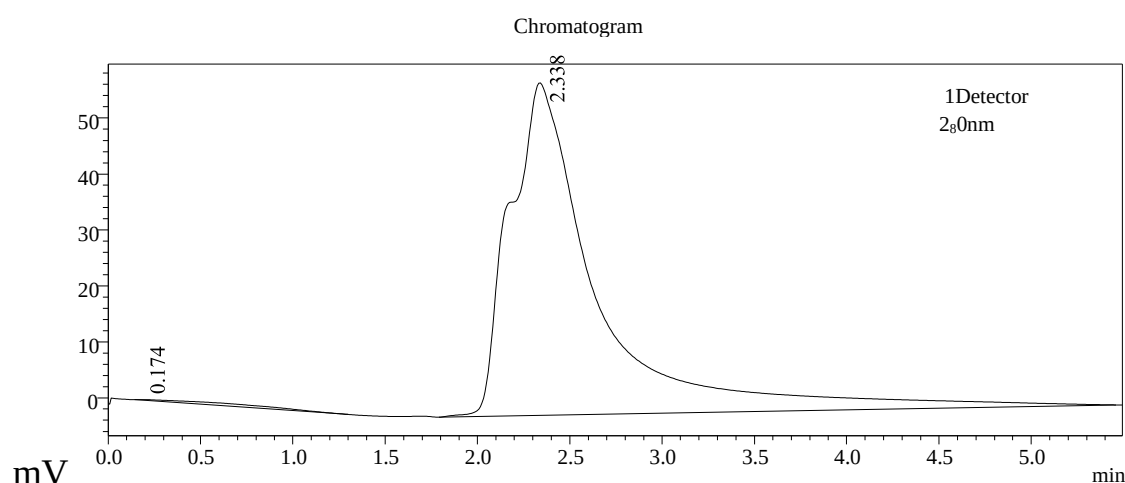


Figure 2: Standard curve used to estimate the approximate molecular weight of phoenixin-14 using gel filtration technique.

The approximate concentration of PNX-14, which was partially purified from the serum of obes women with PCOS, was determined by high-performance liquid chromatography (HPLC):

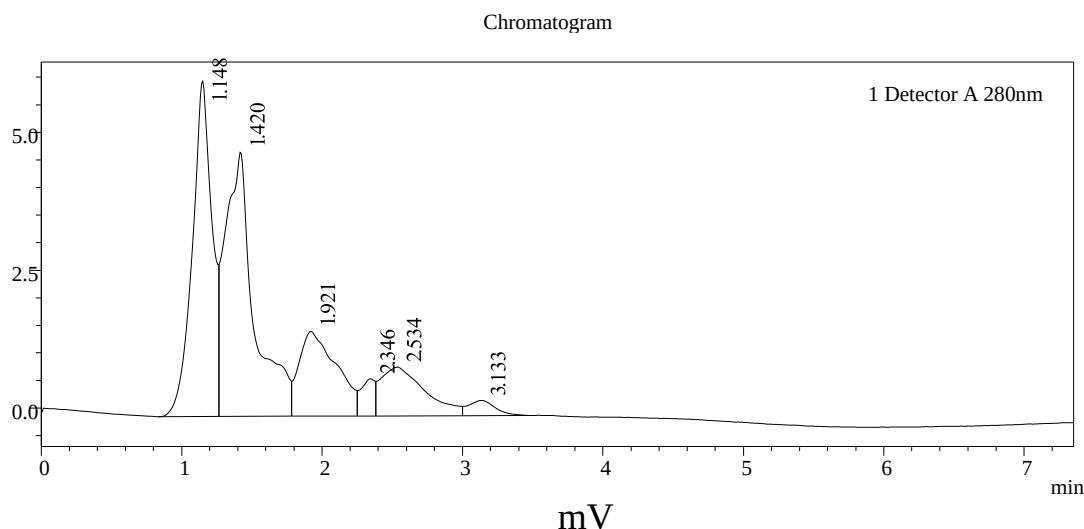
The results of using the HPLC technique used to determine the purity of the hormone PNX-14 (peak A) isolated and purified from the blood serum of obes women with PCOS by gel filtration chromatography technique (Sephadex G-25) [26]. The results shown in Figure (3) indicate that there are four bands, the third band (of the main band) at the minute (2.338).



Figure(3): Chromatogram standard solution for PNX-14.

he results obtained from Figure (4) showed that six bands appeared after injecting the phoenixin-14 solution (peak A) isolated and purified from the blood serum of

obese women with polycystic ovary syndrome by gel filtration technique in the column of a high-performance liquid chromatography device reversed phase under standard conditions for the solution benchmark. The fourth package (peak A) at (2.346) minutes.



Figur(4): Chromatograms of PNX-14 (peak A) Isolated and Purified from the Serum of PCOS Patients

When comparing the standard phoenixin-14 chromatogram with the isolated and purified phoenixin-14 (peak A) chromatogram, it was found that there was a good similarity between the retention time of standard pheonixin-14 with the retention time of phoenixin-14 (peak A) isolated and purified from the blood serum of obese women with PCOS. This comparison indicates that phoenixin-14 (peak A) isolated and purified from the serum of infected women with purification steps is almost identical to the properties of standard phoenixin-14.

Conclusion:

The increased concentration of phoenixin-14 hormone in the serum of obese women with polycystic ovary syndrome is a very good biomarker for diagnosing the syndrome compared to healthy people.

A significant difference was found in the concentration of PNX-14 in the blood serum of obese women with PCOS. The approximate molecular weight was mcalculated by gel filtration chromatography calculated by gel filtration chromatography (1697 mol/L), but when using the (HPLC) technique, We found that the retention time (2.338) of standard funkycin-14 is similar to the retention time (2.346) of funkycin-14 (peak A) isolated and purified from the blood serum of women with PCOS.



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